THE CONTENT OF ASCORBIC ACID IN THE ADRENAL GLANDS AND THE PRODUCTION OF CORTICOSTEROIDS IN VITRO IN IRRADIATED, IMMOBILIZED, AND HYPOPHYSECTOMIZED RATS

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THE CONTENT OF ASCORBIC ACID IN THE ADRENAL GLANDS AND THE PRODUCTION OF CORTICOSTEROIDS IN VITRO IN IRRADIATED, IMMOBILIZED, AND HYPOPHYSECTOMIZED RATS

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Our preceding investigations showed that in the acute period of /66** radiation sickness, in rats, the reaction of the hypophysis-adrenal system to the effect of extremely powerful stimuli does not significantly differ from the reaction of normal animals to the primary effect of these factors. In connection with the data obtained, it became necessary to clarify the functional condition of the adrenal cortex in rats at the peak of acute radiation injury. Reports available in the literature on this question proved to be contradictory. On the one hand [1 - 3], there were indications of exhaustion of adrenal function during radiation sickness, while on the other — a number of authors [4 - 6] did not find signs of significant suppression in the processes of corticosteroid formation and secretion.

In order to answer the question posed, we studied the content /67 of ascorbic acid in the adrenal glands (as one of the indirect indices of functional activity of the adrenal cortex), and also their

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capacity to produce corticosteroids under conditions of incubation in experiments in vitro.

Materials and Methods

The investigations were conducted in 2 series on 4 groups of male rats: irradiated, hypophysectomized, immobilized, and control groups. The first group was made up of rats irradiated with a lethal dose of x-rays (800 r). The conditions of irradiation were standard. The rats were killed by decapitation on the 5th - 8th day after irradiation. In this period, a pronounced clinical picture of acute radiation sickness developed. Body weight and the number of leucocytes in the peripheral blood were recorded in the animals. After they were killed, the weight of the thymus and adrenal glands was recorded. The second group consisted of hypophysectomized rats. The operation was conducted via the paratracheal pathway [7], 48 hours and 2 weeks prior to the experiment. The animals of the third group were tied to a stand upside down for 1 hour, after which they were killed. The rats of the control group (fourth group) were killed immediately after their removal from the cage.

In the first series of experiments, the content of ascorbic acid in the left adrenal gland was determined in the animals of all four groups after the method of Roe and Kuether [8], in the Geschwind modification [9].

In the second series of experiments, both adrenals were removed from the animals for estimating the amount of corticosteroids formed under conditions of their incubation, by the method of Saffran and Schally [10] in experiments in vitro. Eighty rats were used in this series. The adrenals of each rat were purified of adipose tissue and were divided into four parts. The eight adrenal quarters combined from each rat were weighed on torsion scales, and were placed in a vessel containing 1.5 ml Krebs-Ringer-bicarbonate-glucose medium with a pH of 7.2 - 7.4. The adrenals were incubated for a one-hour period in a Warburg apparatus in an atmosphere containing 95% oxygen and 5% carbon dioxide gas. From 1 ml incubate, the

steroids were extracted with 3 ml methylene chloride by intensively shaking the test tubes for two minutes. During this process, an emulsion formed from which samples were separated by centrifuging at 2000 rpm for 5 minutes. The incubating medium (upper layer) was removed by suction with a syringe. The methylene chloride with the extracted steroids (lower layer) was poured into another centrifuge test tube and stored in a refrigerator until the next day, when the samples were spectrophotometrically measured on the SF-4. Quantitative determination of steroids was carried out by two methods: according to the difference in optical densities (D) of the extract at 240 and 255 millimicrons, and according to the difference in these indices relative to the weight of the adrenals [10]. Subsequently, the methylene chloride was evaporated and a tetrazole blue reaction was set up with the steroid precipitate [11, 12]. For this purpose, 0.1 ml 1% alcohol solution tetramethyl-ammonium-hydroxide and 0.1 ml 0.40% alcohol solution tetrazole blue were added to the dry precipitate. The test tubes were placed for 30 minutes in an incubator at a temperature of 28°. 0.1 ml ice-cold acetic acid and 1.2 ml freshly distilled ethyl alcohol were added to the sample. The color intensities of the farmazan formed as the result of reduction by the steroids, which have an α-ketol side chain, and of the tetrazole blue, were measured on the colorimeter using a green filter. The calculation was carried out according to an earlier complied curve of standard solution of crystalline corticosterone. The amount of corticosteroids formed was expressed in micrograms per hour and in micrograms per 100 mg adrenal tissue per hour.

A number of investigations [13, 15] emphasize that methylene chloride primarily extracts steroids, and the main product formed by the adrenal tissue of rats is corticosterone.

Based on these data, one could hypothesize that in our experiments the quantitative changes, predominantly of corticosterone, are evaluated. The results of the investigations conducted confirm this hypothesis. After chromatography of the extract, a spot of steroid on the chromatogram was found in the location of

standard corticosterone. R_f of the spot in the system used by us (heptane-benzene-methanol-water) was 0.1, which corresponds to indications in the literature [16].

Results

The investigations carried out on immobilized and hypophysectomized rats had the goal of clarifying the level of production of corticosteroids in experiments in vitro in animals with previously altered functional activity of the adrenal cortex. It is known that the function of the adrenal cortex is stimulated under the influence of ACTH and, on the other hand, is inhibited in the absence of ACTH. To stimulate the secretion of endogenic ACTH from the hypophysis, we immobilized the animals. Immobilization is a powerful stimulus and has been used by a number of authors [17 - 20] as a model for studying various problems of the physiology and patho-physiology of the hypophysis-adrenal system. With the goal of suppressing the function of the adrenals, we employed surgical hypophysectomy. /68

Data obtained by means of determining the content of corticosteroids in the blood of the suprarenal vein, the peripheral blood or the urine, and also the indirect indices — the concentration of cholesterol and ascorbic acid of the adrenals are considered widely accepted indices of the functional condition of the adrenal glands.

In our previous investigations it was shown that secretion of ACTH from the hypophysis is always accompanied by a decrease in the level of ascorbic acid in the adrenals. In our experiments, immobilization of the animal for one hour led to a clear-cut decrease in the ascorbic acid content of the adrenals (Table 1). The concentration of ascorbic acid per 100 g adrenal tissue also decreased, inasmuch as immobilization did not cause a noticeable change in the weight of the adrenals.

TABLE 1. CONTENT AND CONCENTRATION OF ASCORBIC ACID IN THE ADRENAL GLANDS OF RATS*

Group of animals	Body weight (g)	Thymus wt (mg) M ± m	Left adrenal wt (mg)	P	Ascorbic acid content (mlg)	P	Ascorbic acid conc. (mg %)	P
Control	179—190 (*n*-38)	330±27	15,3±0,4		60,0±2,6		386±9	
3rd	170 (*n*-13)	—	14,2±0,8	0,25	45,7±3,0	<0,001	321±10	<0,001
2nd (operation 14 days prior to experiment)	138 (*n*-16)	—	5,4±0,3	<0,001	33,5±2,1	<0,001	649±49	<0,001
1st	182—154 (*n*-34)	82±5	20,9±0,8	<0,001	76,1±3,3	<0,001	379±7	>0,5

TABLE 2. PRODUCTION OF CORTICOSTEROIDS BY RAT ADRENALS, ACCORDING TO SPECTROPHOTOMETRIC DATA AND REACTION WITH TETRAZOLE BLUE*

Groups of animals	Body wt (g)	Thymus wt (mg)	Wt of both adrenals (mg)	From spectrophotometric data					From tetrazole blue reaction			
				P	$\frac{D\ (240-255)\times}{\times 1000}$	P	$\frac{D\ (240-255)\times 10}{\text{wt. of adrenals}}$	P	mcg/hr	P	mcg per 100 mg adrenal tissue per hr	P
Control	174 (*n*-37)	321	25,0±0,6		43±2,0		16,9±0,9		8,0±0,7		32,7±3,0	
3rd	173 (*n*-13)	—	25,2±1,3	>0,5	64±2,5	<0,001	24,0±1,2	<0,001	14,6±2,2	=0,002	56,7±8,3	<0,002
2nd (operation 14 days prior to experiment)	140 (*n*-10)	—	10,4±0,3	<0,001	22±1,7	<0,001	21,1±1,8	<0,05	4,0±0,2	<0,001	38,4±1,4	=0,1
1st	184—155 (*n*-15)	82	41,9±2,7	<0,001	66±4,6	<0,001	16,0±1,2	=0,5	17,2±1,5	<0,001	41,6±4,7	=0,1

*Translator's note: Commas represent decimal points.

When determining the amount of corticosteroids formed by the adrenal tissue of the immobilized animals, it turned out that the adrenals of these rats produce significantly more corticosteroids in vitro than the adrenals of the control animals under the same conditions (Table 2). When calculating the obtained values per 100 mg adrenal tissue, a significant and statistically reliable difference was also noted. In the immobilized rats of this series of experiments, as in the mentioned group of rats of the first series, no change in the weight of the adrenals was observed.

When comparing the results of determining ascorbic acid and biosynthesis of steroids by the adrenals, we see that in the immobilized animals the decrease in the amount and concentration of ascorbic acid in the adrenals is accompanied by a simultaneous increase in the production of corticosteroids in the experiments in vitro. Since the weight of the adrenals does not significantly change in the process of immobilization, the increased production of steroids indicates an increase in the biosynthetic capacities of the adrenal tissue as the result of immobilization.

In the hypophysectomized animals, the weight of the adrenals and the amount of ascorbic acid in them decreased. However, the concentration of the latter was higher during this process (649 mg %), since the weight of the adrenals decreased nearly two-fold two weeks after the operation (see Table 1). During incubation of the adrenals of the hypophysectomized rat, suppression of steroid production of almost two-fold in comparison with the norm (see Table 2) was noted.

The obtained data indicate significant suppression of the process of steroid biosynthesis by the adrenals of hypophysectomized rats in experiments in vitro.

A number of authors [22, 23], when determining the functional activity of the adrenals in experiments in vivo, found an extremely low content of corticosterone in hypophysectomized rats in the peripheral blood, and in the blood flowing from the adrenals, while

in the immobilized animals, on the other hand, a high level of corticosterone was found. In comparing these data with the results of calculating the amount of corticosteroids produced by the adrenals of hypophysectomized and immobilized rats in experiments in vitro obtained by us, we concluded that the production of corticosteroids determined under conditions of incubating the adrenal tissue in experiments in vitro entirely reflects the functional activity of this gland in the organism of the animal. Having convinced ourselves of this, we conducted investigations on irradiated rats.

On the 4^{th} - 8^{th} day after whole-body irradiation, the weight of the animals fell; there was a sharp decrease in the weight of the thymus (almost 4-fold); there was a decrease in the number of leucocytes in the peripheral blood, and sharp hypertrophy of the adrenals was noted. The weight of the adrenals in some rats increase 2 - 2 1/2 times, in comparison with the norm. During this process, there was also an increase in their content of ascorbic acid (see Table 1). However, its concentration in the gland did not differ from the concentration in the control. /70

When incubating the adrenals of the irradiated rats, it was found that the production of corticosteroids clearly increased (see Table 2). When calculating the amount of steroids produced per 100 mg adrenal tissue in the irradiated rats and normal rats, no difference was found.

Hence, in the irradiated animals, a sharp increase was observed in the weight of the adrenals, and there was a corresponding increase as well in the amount of ascorbic acid in them and in the production of corticosteroids during incubation of the animal's adrenals in vitro at the peak of radiation sickness. However, the biosynthetic capacity of the adrenal tissue per unit of weight did not differ from the norm. The results obtained indicate an increase in the functional activity of the adrenals of the irradiated animal at the peak of acute radiation sickness.

It was of interest to clarify whether this reaction was an intermediary one similar to the early reaction of the adrenals to radiation through the hypophysis. With this goal, 23 rats were subjected to hypophysectomy. Forty-eight hours after the operation, 18 of the animals were irradiated with a dose of 800 r, while 5 served as the control. On the third day after irradiation, 9 of the 18 rats died, while of the 12 irradiated non-hypophysectomized rats, only 1 died in this period of time. On the fifth day after hypophysectomy, the 5 control animals and the 9 irradiated animals which had survived were killed.

No increase in the weight of the adrenals was observed in the hypophysectomized rats on the third day after irradiation. The production of corticosteroids in the adrenals of the hypophysectomized, irradiated rats went on at the same level as the production of steroids in the adrenals of the hypophysectomized rats not subjected to irradiation (Table 3).

The results obtained show that adrenal hypertrophy and the consequent increased production of corticosteroids are caused by ACTH secreted by the hypophysis. It is interesting to note that there was also a decrease in thymus weight in the absence of hypophysis in the irradiated rats, which was apparently caused not only by the effect of ACTH, but also by the direct effect of ionizing radiation on this gland.

In summarizing these data, one can say that the relationship between the production of corticosteroids and the reaction of adrenal ascorbic acid, in all probability, exists under conditions of only short-term, acute stress, while under conditions of long-term stress leading to a change in the weight of the adrenals (radiation sickness), this relationship is disrupted. It should be noted that with a certain experimental set-up, some authors [24, 25] also failed to find a relationship between corticosteroidogenesis and the concentration of ascorbic acid in the adrenals. /71

TABLE 3. PRODUCTION OF CORTICOSTEROIDS BY THE ADRENAL GLANDS OF HYPOPHYSECTOMIZED, IRRADIATED AND HYPOSECTOMIZED NON-IRRADIATED RATS*

Group of animals	Body weight (in g)	Thymus weight (in mg)	Weight of both adrenals (in mg)	From spectrophotometric data		From reaction with tetrazole blue	
				D (240-255)×1000	$\frac{D\ (240-255)\times 1000}{\text{Wt. of adrenals}}$	mcg/hour	mcg per 100 mg adrenal tissue per hour
Control (hypophysectomized, non-irradiated)	199—180 (*n*-5)	392	15,2	20	14,4	6,0	42,0
Experimental (hypophysectomized, irradiated)	197—170 (*n*-9)	106	14,9	22	17,0	5,9	33,7

*Translator's note: Commas represent decimal points.

Conclusions

1. In rats immobilized for a period of 1 hour, the content and concentration of ascorbic acid in the adrenals decrease, while the production of corticosteroids increases. There is an increase in the biosynthetic capacity of the adrenal tissue. The weight of the adrenal glands does not change.

2. Two weeks after hypophysectomy, there is a sharp increase in the weight of the adrenals and in the amount of ascorbic acid in them in the hypophysectomized rats. The concentration of ascorbic acid increases. Production of corticosteroids decreases in experiments in vitro.

3. In irradiated rats, the weight of the adrenal glands and their ascorbic acid content increase in the peak period of radiation sickness. The concentration of ascorbic acid does not change. Production of corticosteroids by the adrenal glands increases in experiments in vitro. The changes do not develop in hypophysectomized animals under the influence of radiation.

4. A direct relationship is noted between the concentration of ascorbic acid in the adrenal glands of immobilized and hypophysectomized rats and the capacity of this gland to produce corticosteroids. No such relationship is observed in the irradiated animals.

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16. Abstract

In rats immobilized for a period of one hour the content and concentration of ascorbic acid in the adrenal glands fell, whereas corticosteroid production — intensified. There was a rise of biosynthetic capacity of the adrenal tissue. No change occurred in the weight of the adrenal glands under the effect of immobilization.

In hypophysectomized rats, 2 weeks after the operation a marked reduction of the adrenal gland weight and ascorbic acid content in them was noted. Ascorbic acid content rose, and corticosteroid production exhibited a drop in experiments in vitro.

At the height of radiation sickness in irradiated rats there was a rise of the adrenal gland weight and an increase of ascorbic acid content in them. Ascorbic acid content failed to change, and corticosteroid production increased in vitro. These changes did not develop in hypophysectomized animals.

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